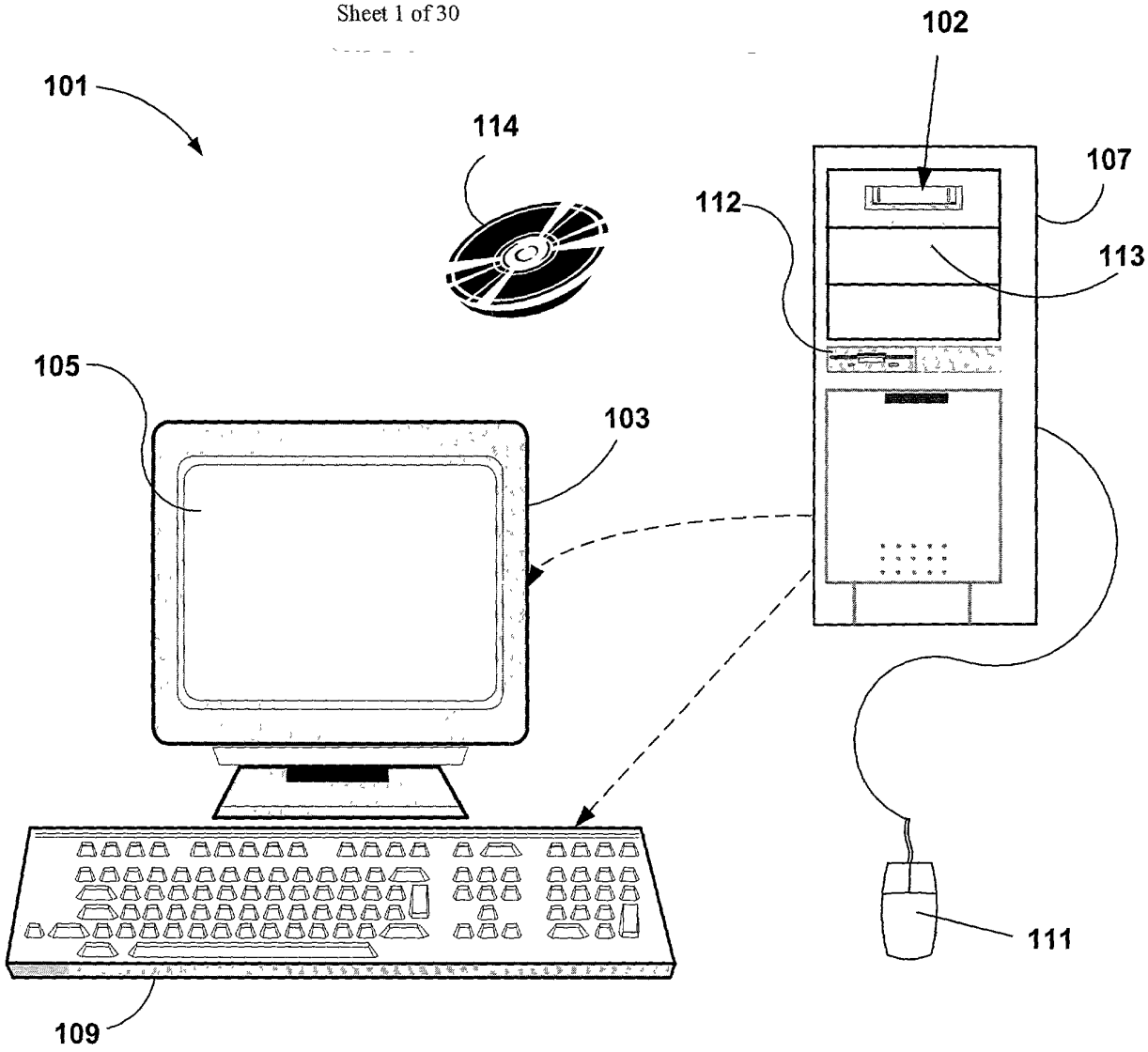


Figure 1

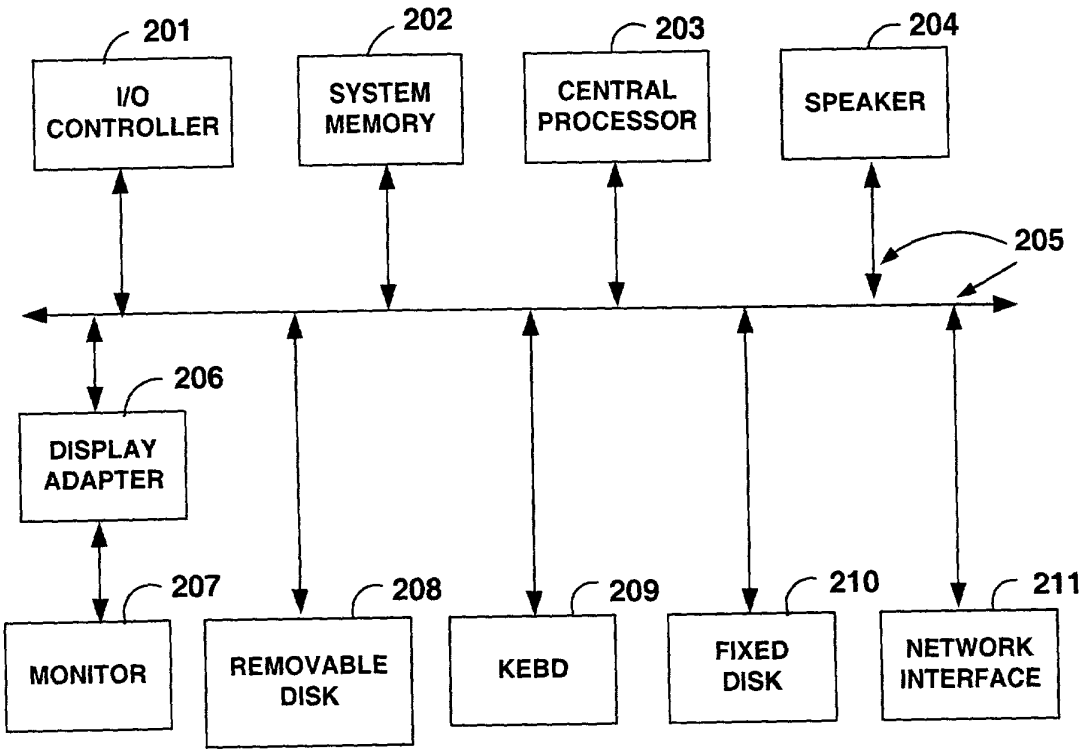
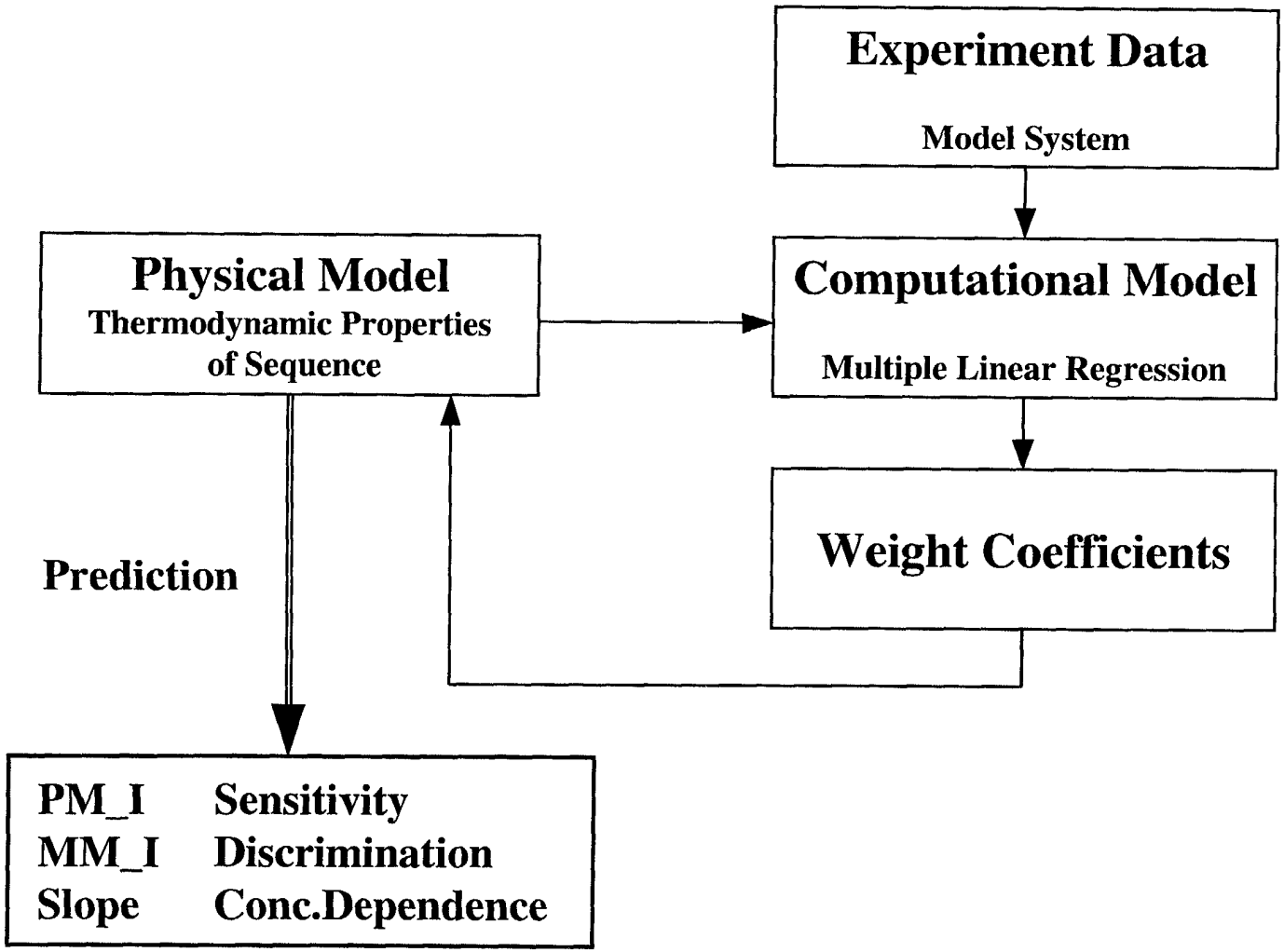

201
I/O CONTROLLER
202
SYSTEM MEMORY
203
CENTRAL PROCESSOR
204
SPEAKER
205
206
DISPLAY ADAPTER
207
MONITOR
208
REMOVABLE DISK
209
KEBD
210
FIXED DISK
211
NETWORK INTERFACE

Figure 2

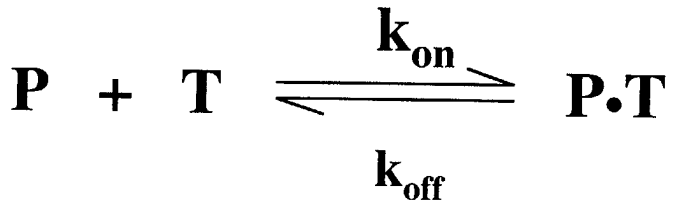
Predicting Probe Quality

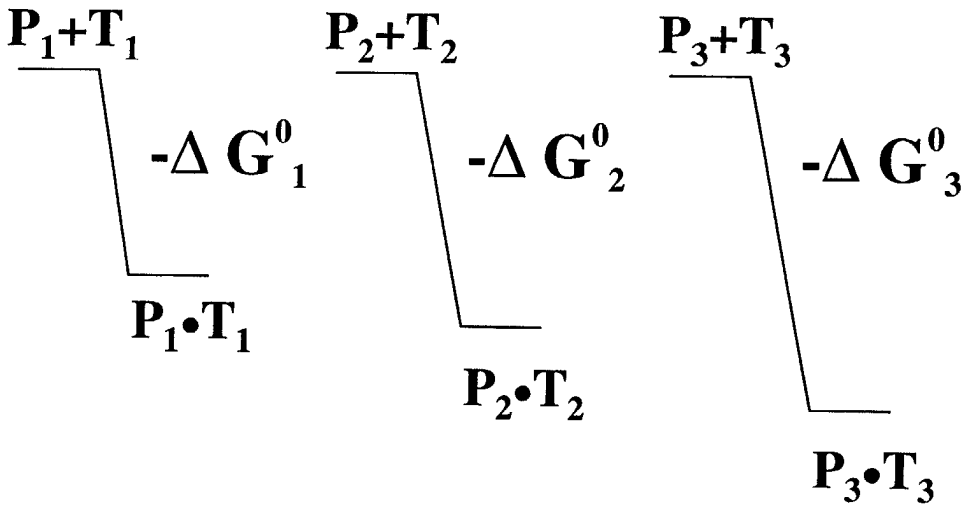
Figure 3



Basic Physical Model

Figure 4





Define Each Nucleotide at Each position

Figure 5

Example : GTCA

***Using A as ref. 3 base/position**

i	Position	Base	S_i	
1	1	C	0	
2	1	G	1	(1st position is G)
3	1	T	0	
4	2	C	0	
5	2	G	0	
6	2	T	1	(2nd position is T)
7	3	C	1	(3rd position is C)
8	3	G	0	
9	3	T	0	
10	4	C	0	(4th position is A as reference)
11	4	G	0	
12	4	T	0	

Title: Methods for Selecting Nucleic Acid Probes
Inventor: Hubbell
Attorney Docket No. 3373.1
Sheet 5 of 30

Relative ΔG vs. Base Position

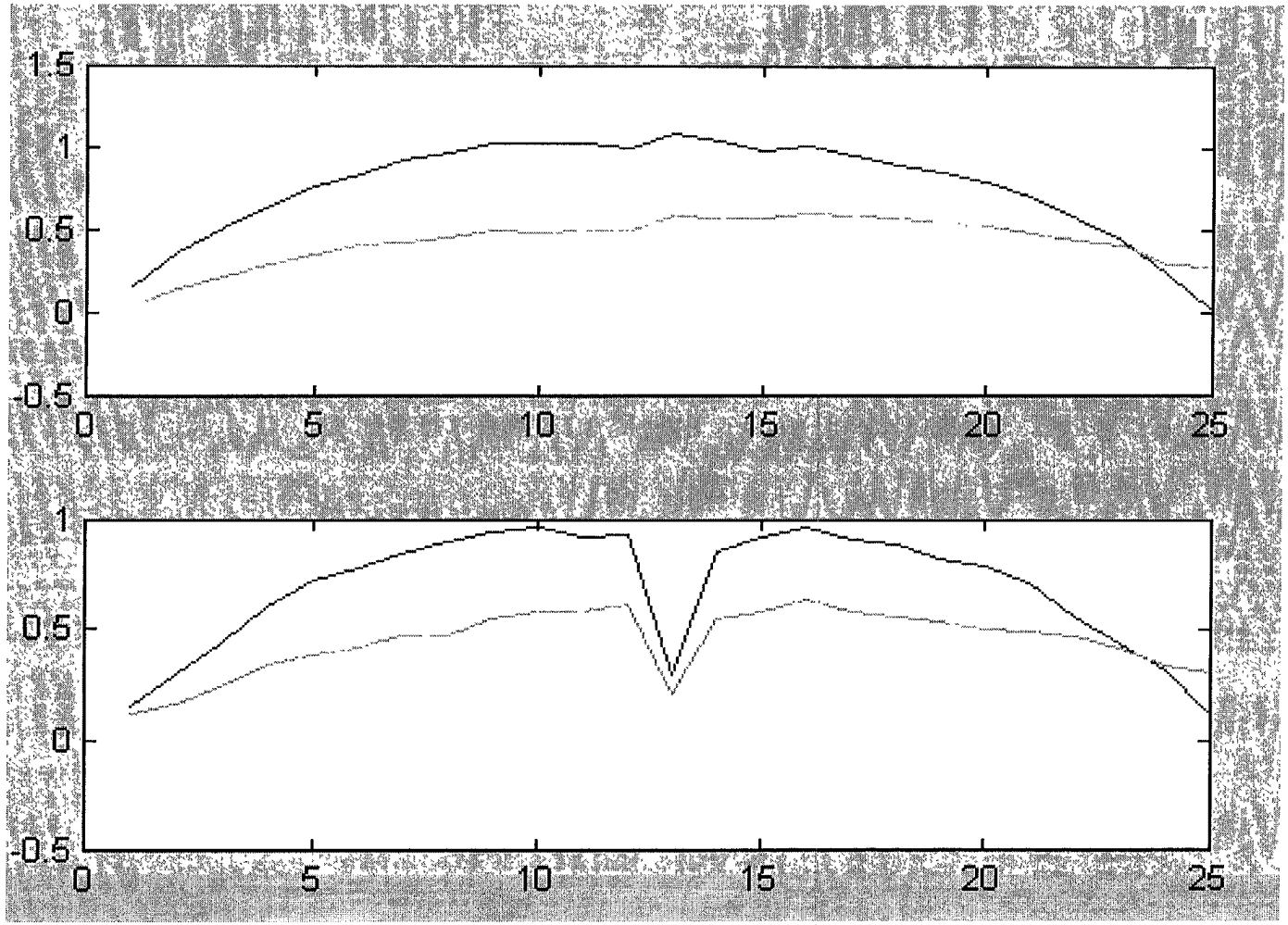


Figure 6A

PM

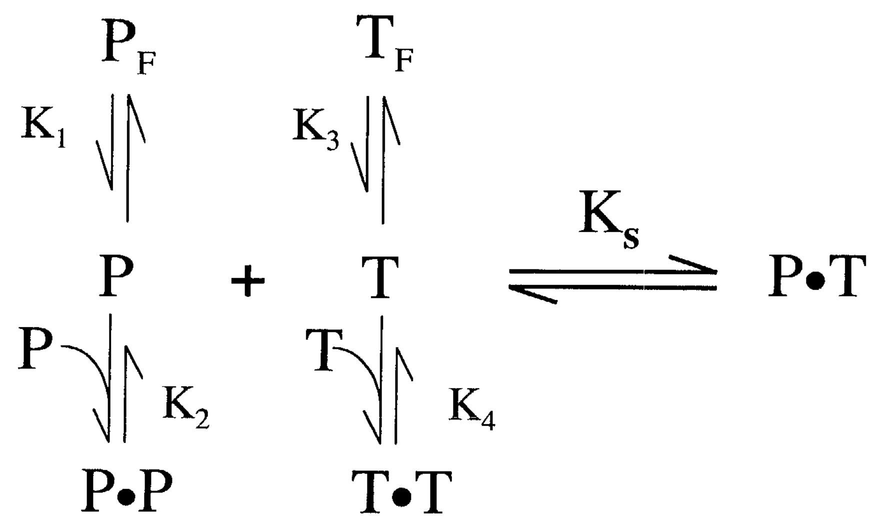
Figure 6B

MM

Base Position in Probe Sequence

Figure 7

Overall Reaction



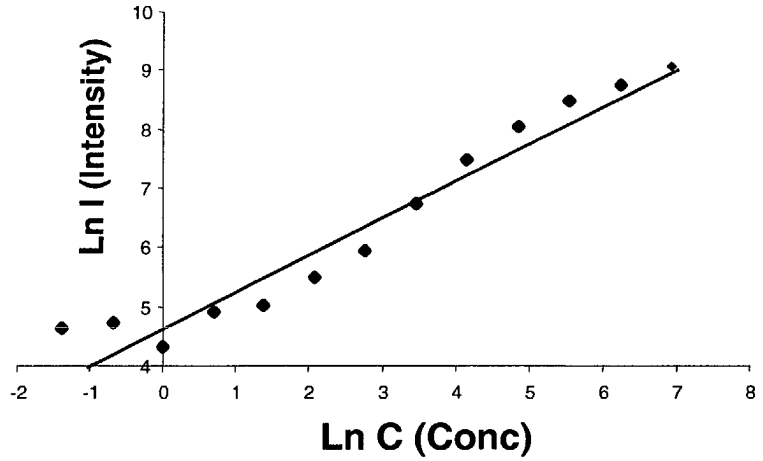
ΔG^0_{PF}: probe folding (K_1)
ΔG^0_{PP}: probe dimerization (K_2)
ΔG^0_{TF}: target folding (K_3)
ΔG^0_{TT}: target interaction (K_4)
ΔG^0_d: duplex formation

Concentration Dependence: Slope

Figure 8

$$\text{Ln } I = S * \text{Ln } C + \text{Ln } K_{app}$$

$$I = K_{app} * C^{S}$$



I: Intensity

K_{app}: Apparent Affinity Constant

C: Concentration

S: Empirical Value ($0 < S < 1$)

Relationship between Kapp vs. S

- Prediction of Probe Saturation

Figure 9A

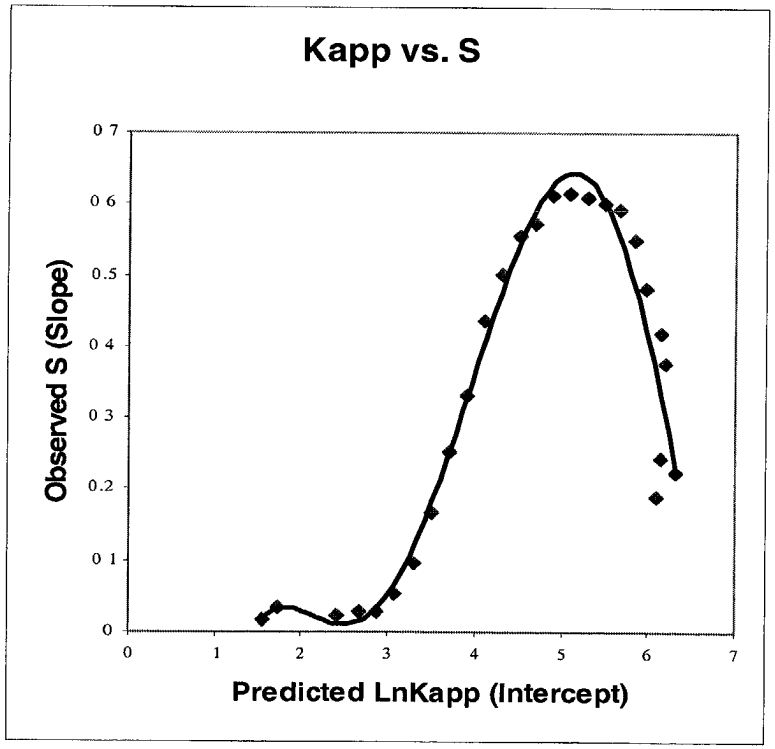
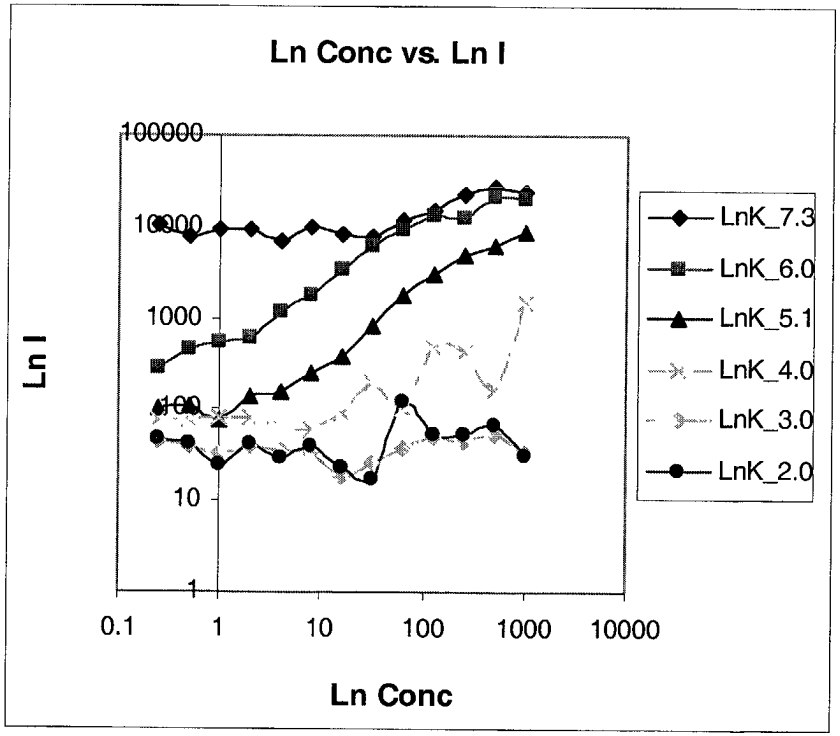


Figure 9B



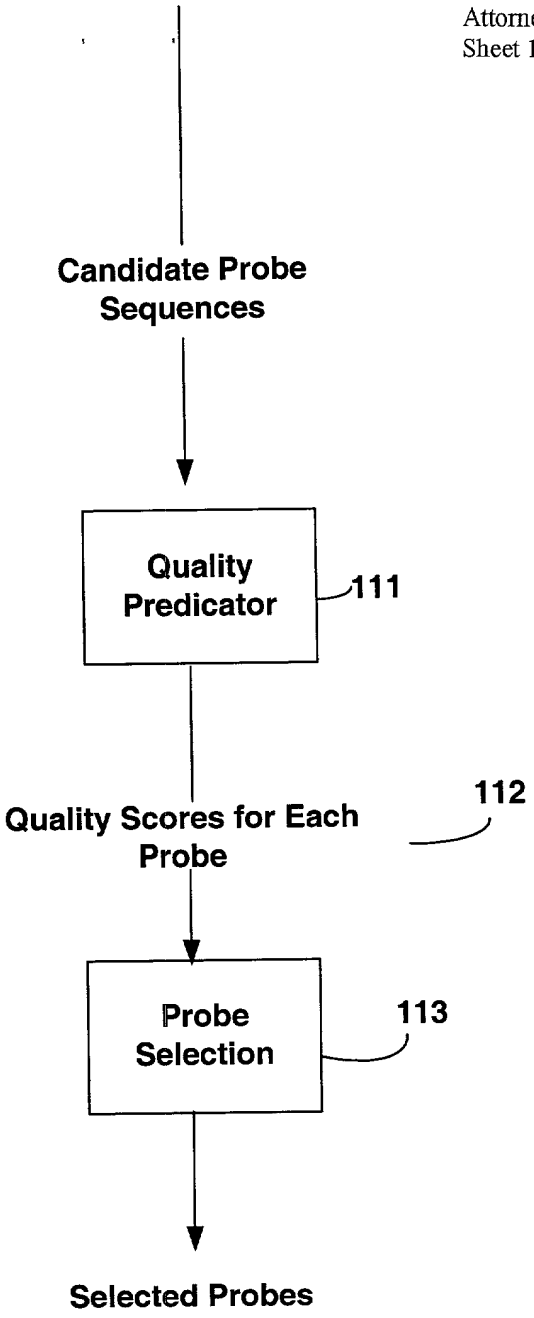

Candidate Probe Sequences
Quality Predicator
111
Quality Scores for Each Probe
112
Probe Selection
113
Selected Probes

Figure 10

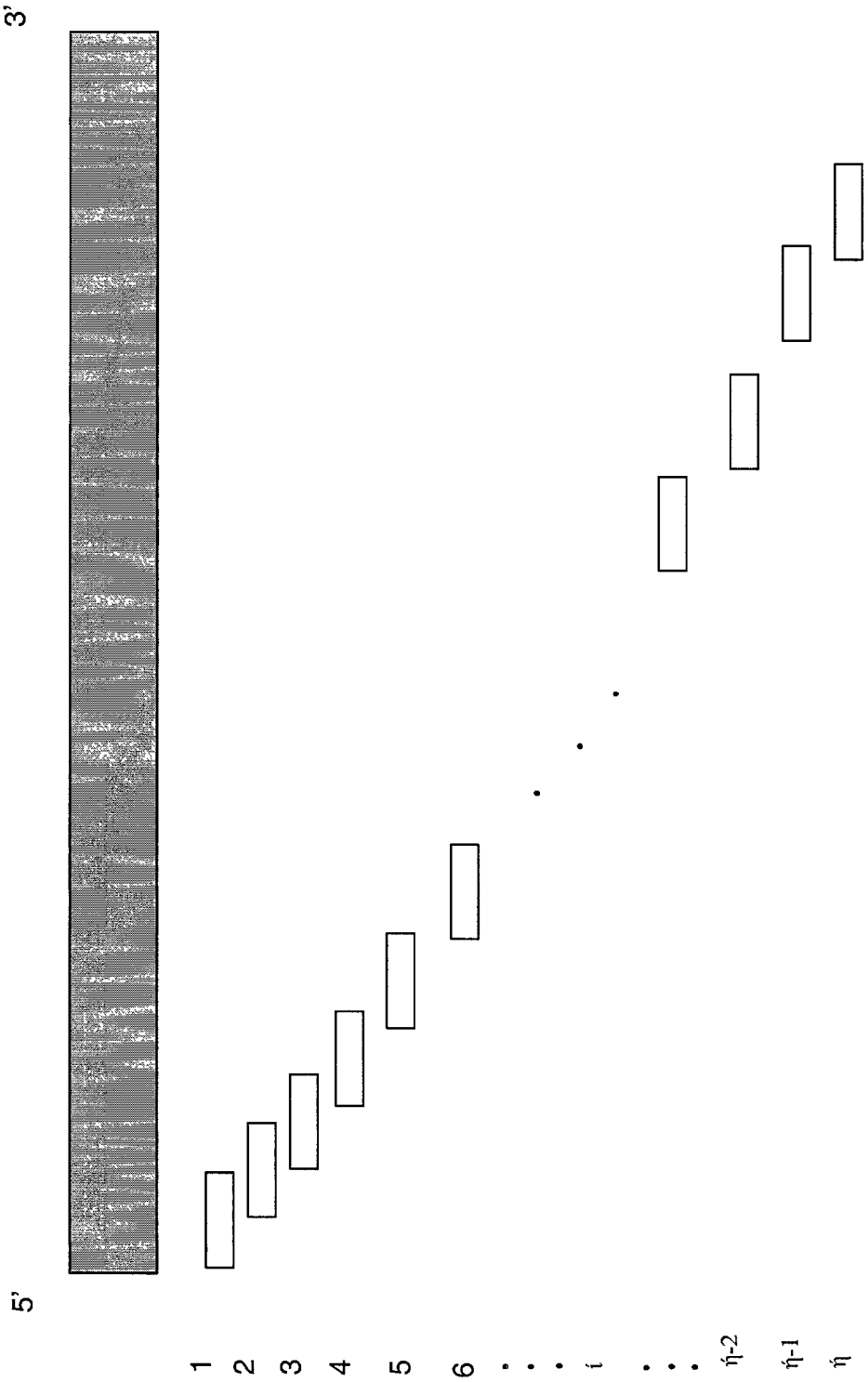


FIGURE 11

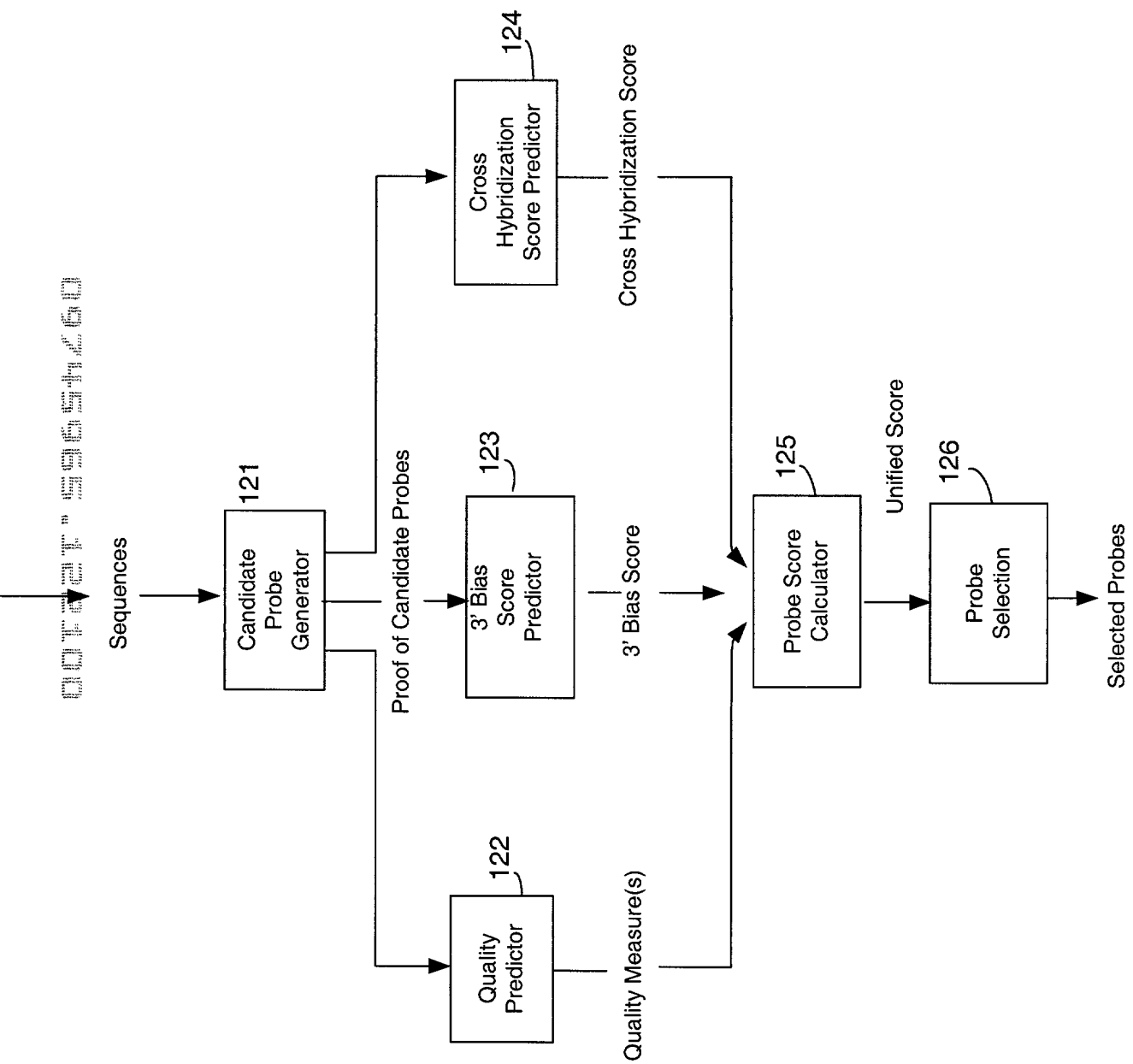

Sequences
Candidate Probe Generator
121
Proof of Candidate Probes
Cross Hybridization Score Predictor
124
Cross Hybridization Score
3' Bias Score Predictor
123
3' Bias Score
Quality Predictor
122
Quality Measure(s)
Probe Score Calculator
125
Unified Score
Probe Selection
126
Selected Probes

Figure 12

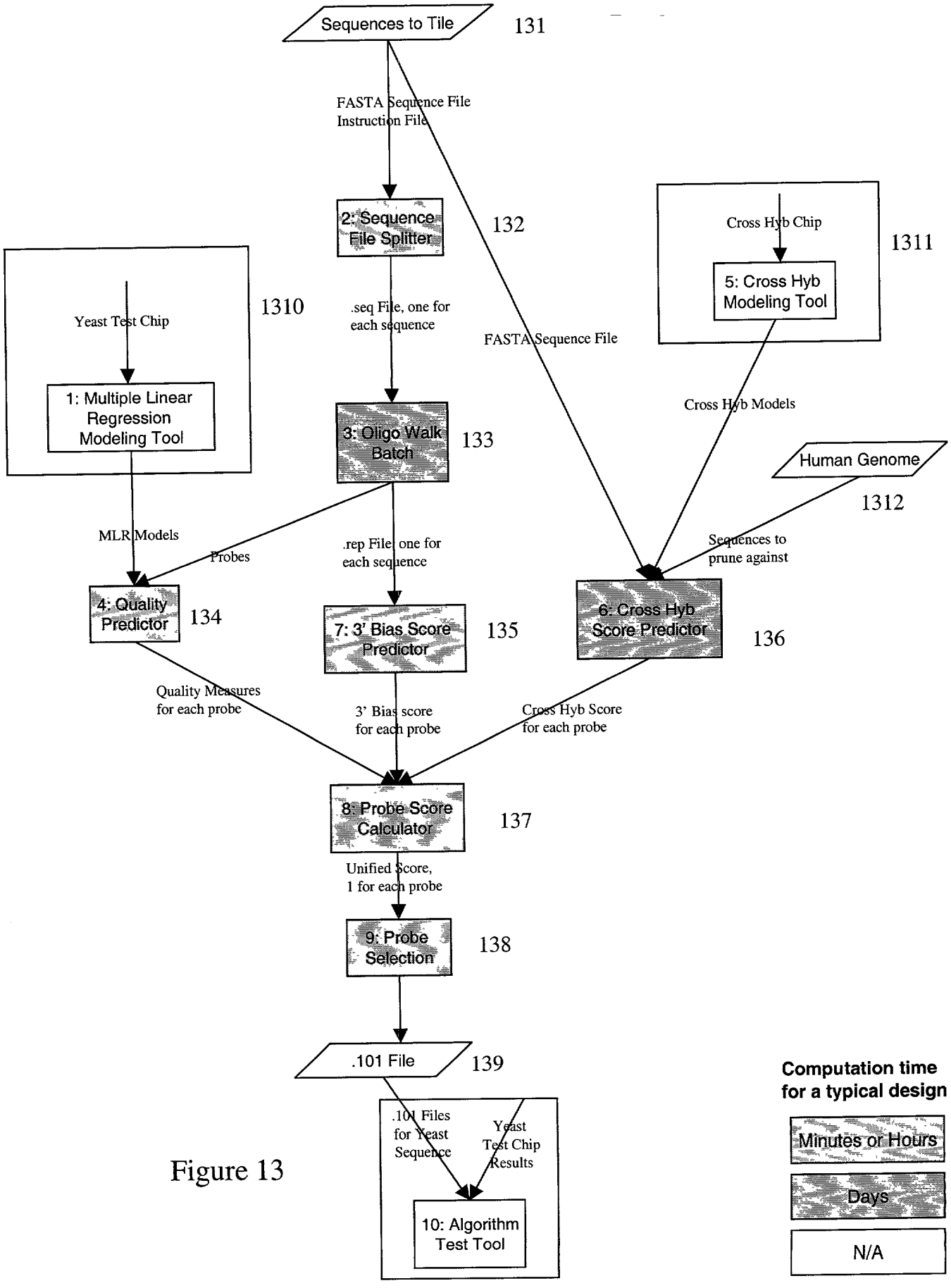

Sequences to Tile
131
FASTA Sequence File
Instruction File
2: Sequence File Splitter
132
Cross Hyb Chip
1311
5: Cross Hyb Modeling Tool
Yeast Test Chip
1310
.seq File, one for each sequence
FASTA Sequence File
1: Multiple Linear Regression Modeling Tool
3: Oligo Walk Batch
133
Cross Hyb Models
Human Genome
1312
MLR Models
Probes
.rep File, one for each sequence
Sequences to prune against
4: Quality Predictor
134
7: 3' Bias Score Predictor
135
6: Cross Hyb Score Predictor
136
Quality Measures for each probe
3' Bias score for each probe
Cross Hyb Score for each probe
8: Probe Score Calculator
137
Unified Score, 1 for each probe
9: Probe Selection
138
.101 File
139
.101 Files for Yeast Sequence
Yeast Test Chip Results
10: Algorithm Test Tool
Computation time for a typical design
Minutes or Hours
Days
N/A

Figure 13

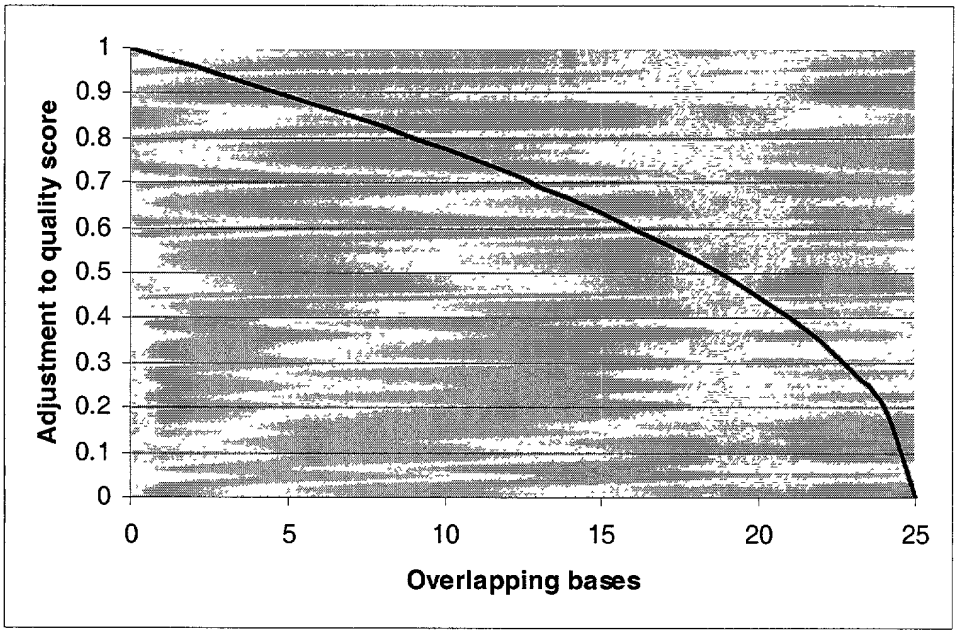


Figure 14

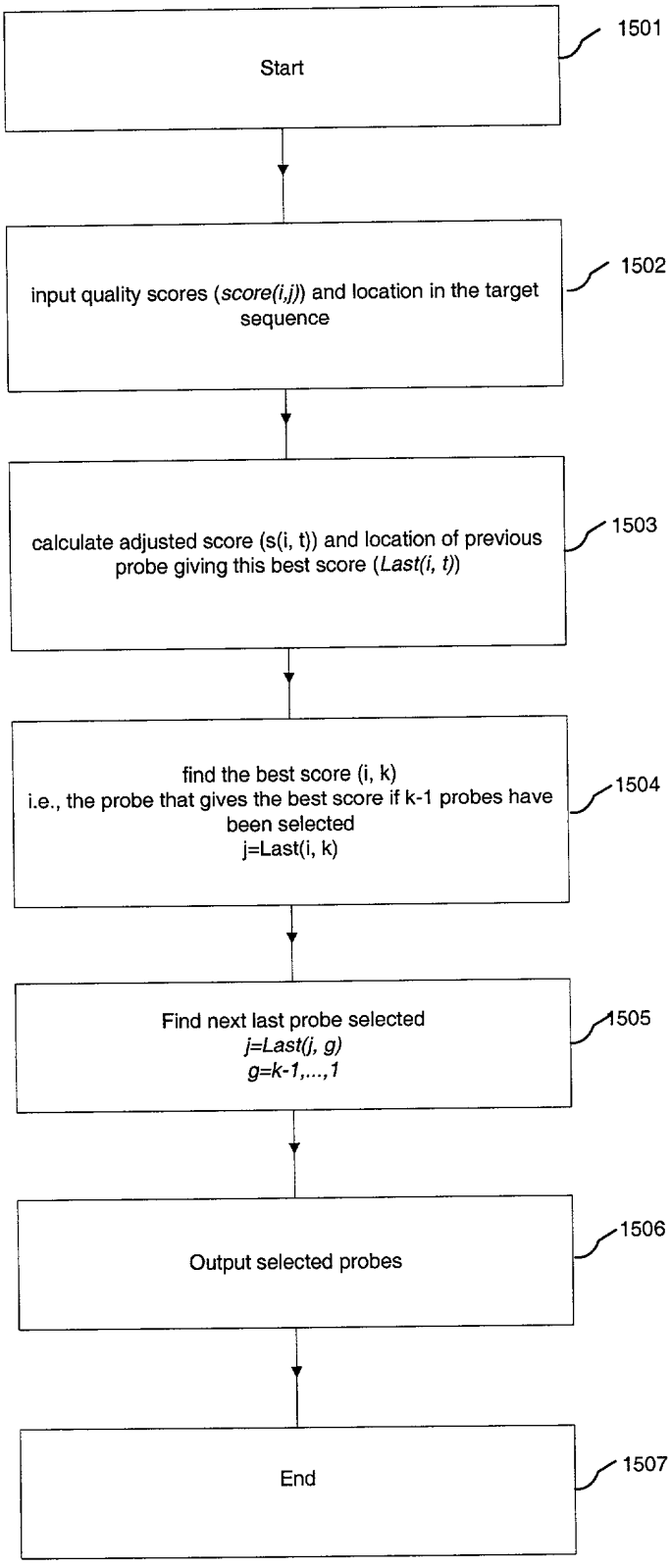

Start
1501
input quality scores (*score(i,j)*) and location in the target sequence
1502
calculate adjusted score (s(i, t)) and location of previous probe giving this best score (*Last(i, t)*)
1503
find the best score (i, k)
i.e., the probe that gives the best score if k-1 probes have been selected
j=Last(i, k)
1504
Find next last probe selected
j=Last(j, g)
g=k-1,...,1
1505
Output selected probes
1506
End
1507

Figure 15

Latin Square MLR

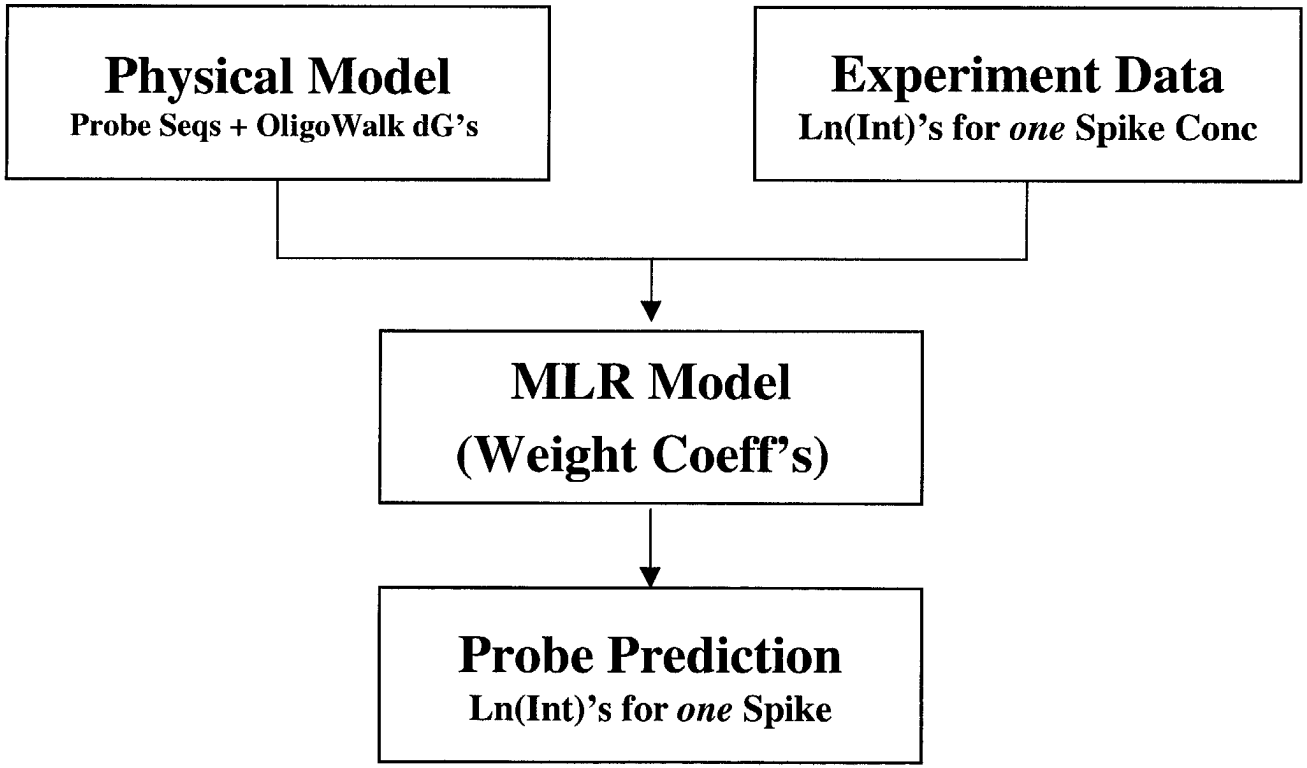


Figure 16

112 Yeast Clones Randomly Divided into 14 Groups

Groups

1	2	3	4	5	6	7	8	9	10	11	12	13	14
YNL259C	YNL037C	YAL038W	YHR044C	YMR127C	YLR377C	YOL064C	YPL209C	YIR034C	YJR148W	YEL046C	YGR185C	YBR166C	YOL165C
YEL003W	YDR113C	YLR083C	YJL117W	YNL290W	YOL086C	YJR094C	YFL029C	YMR276W	YML060W	YGR072W	YGL181W	YJL155C	YNL227C
YDL235C	YGL105W	YLL043W	YMR116C	YMR228W	YJR019C	YIR026C	YGR040W	YMR294W	YDL188C	YMR203W	YGL213C	YEL036C	YNL228W
YEL024W	YDR498C	YBR212W	YPL111W	YPR057W	YOR085W	YLR056W	YPR065W	YPL001W	YGR109C	YGR112W	YOL136C	YJL014W	YMR108W
YEL018W	YDL029W	YNL015W	YCL055W	YNR035C	YDL226C	YMR270C	YPR191W	YFL039C	YOL043C	YHR208W	YEL037C	YJL110C	YPL043W
YER161C	YKL081W	YDL075W	YFR025C	YCL032W	YBL016W	YBR018C	YMR139W	YNL307C	YLR291C	YIL136W	YHL022C	YFL056C	YLR153C
YKL193C	YFR053C	YML098W	YLR354C	YIL154C	YBL068W	YBR057C	YPR035W	YGL148W	YDR088C	YOR099W	YHL014C	YJR155W	YPR074C
YPR129W	YFL018C	YOL143C	YPL069C	YBR034C	YHR025W	YER118C	YNL005C	YGL155W	YNR015W	YOR176W	YKR061W	YNL331C	YPL089C

Figure 17

Title: Methods for Selecting Nucleic Acid Probes
Inventor: Hubbell
Attorney Docket No. 3373.1
Sheet 17 of 30

Latin Square Experiment

Groups →

Exp →

Exp	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	0	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024
2	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	0
3	0.5	1	2	4	8	16	32	64	128	256	512	1024	0	0.25
4	1	2	4	8	16	32	64	128	256	512	1024	0	0.25	0.5
5	2	4	8	16	32	64	128	256	512	1024	0	0.25	0.5	1
6	4	8	16	32	64	128	256	512	1024	0	0.25	0.5	1	2
7	8	16	32	64	128	256	512	1024	0	0.25	0.5	1	2	4
8	16	32	64	128	256	512	1024	0	0.25	0.5	1	2	4	8
9	32	64	128	256	512	1024	0	0.25	0.5	1	2	4	8	16
10	64	128	256	512	1024	0	0.25	0.5	1	2	4	8	16	32
11	128	256	512	1024	0	0.25	0.5	1	2	4	8	16	32	64
12	256	512	1024	0	0.25	0.5	1	2	4	8	16	32	64	128
13	512	1024	0	0.25	0.5	1	2	4	8	16	32	64	128	256
14	1024	0	0.25	0.5	1	2	4	8	16	32	64	128	256	512

Figure 18

Latin Square Data Sets from Yeast_Test_Hyb Chips

Lot 1 (9912072)

No Background:	3 Scans	14 chips	(530, PMT=701; 570, PMT=701; 570, PMT=600)
+ Background:	3 Scans	14 chips	(530, PMT=701; 570, PMT=701; 570, PMT=600)

Lot 2 (9910426)

No Background:	1 Scan	14 chips	(570, PMT=600)
No Background:	1 Scan	14 chips	(570, PMT=600)

Lot 3 (9910427)

+ Background:	1 Scan	14 chips	(570, PMT=526)
No Background_Rep1:	1 Scan	14 chips	(570, PMT=526)
No Background_Rep2:	1 Scan	14 chips	(570, PMT=526)

Lot 4 (9913514)

No Background:	1 Scan	14 chips	(570, PMT=526)
+ Background:	1 Scan	14 chips	(570, PMT=526)

Lot 5 (9914059)

+ Background_Rep1:	1 Scan	14 chips	(570, PMT=526)
+ Background_Rep2:	1 Scan	14 chips	(570, PMT=526)
No Background:	1 Scan	14 chips	(570, PMT=526)

Figure 19

Bootstrapping

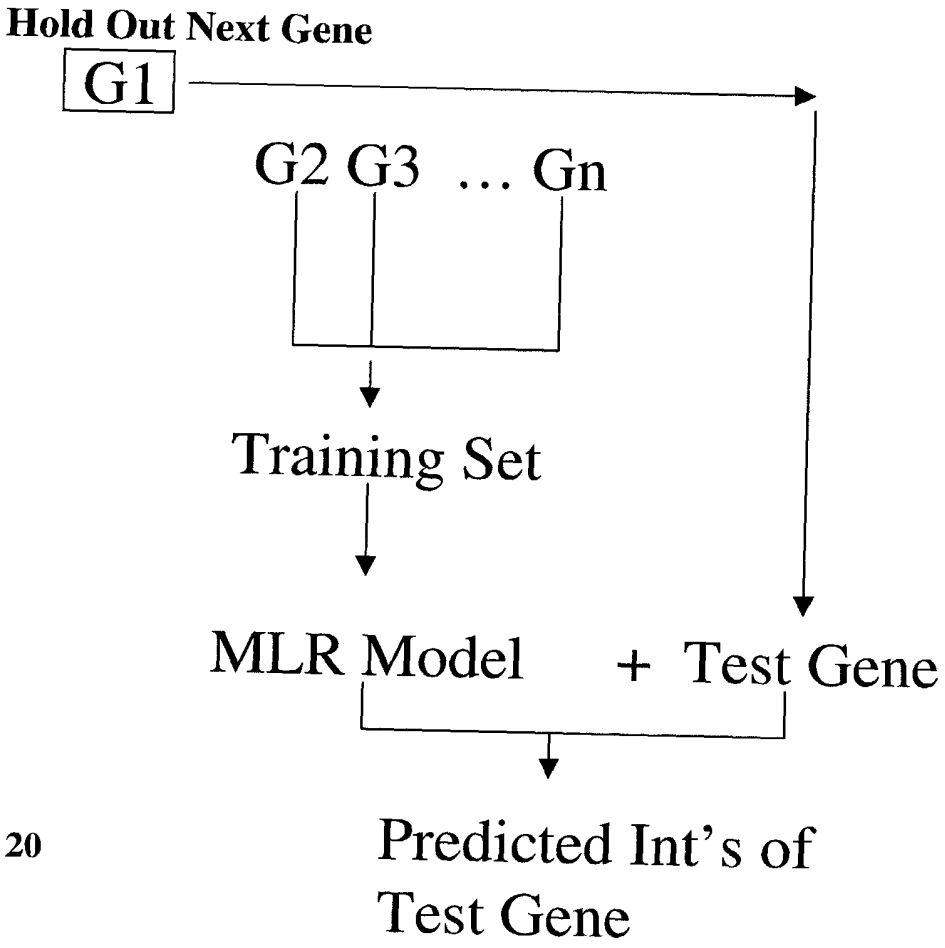


Figure 20

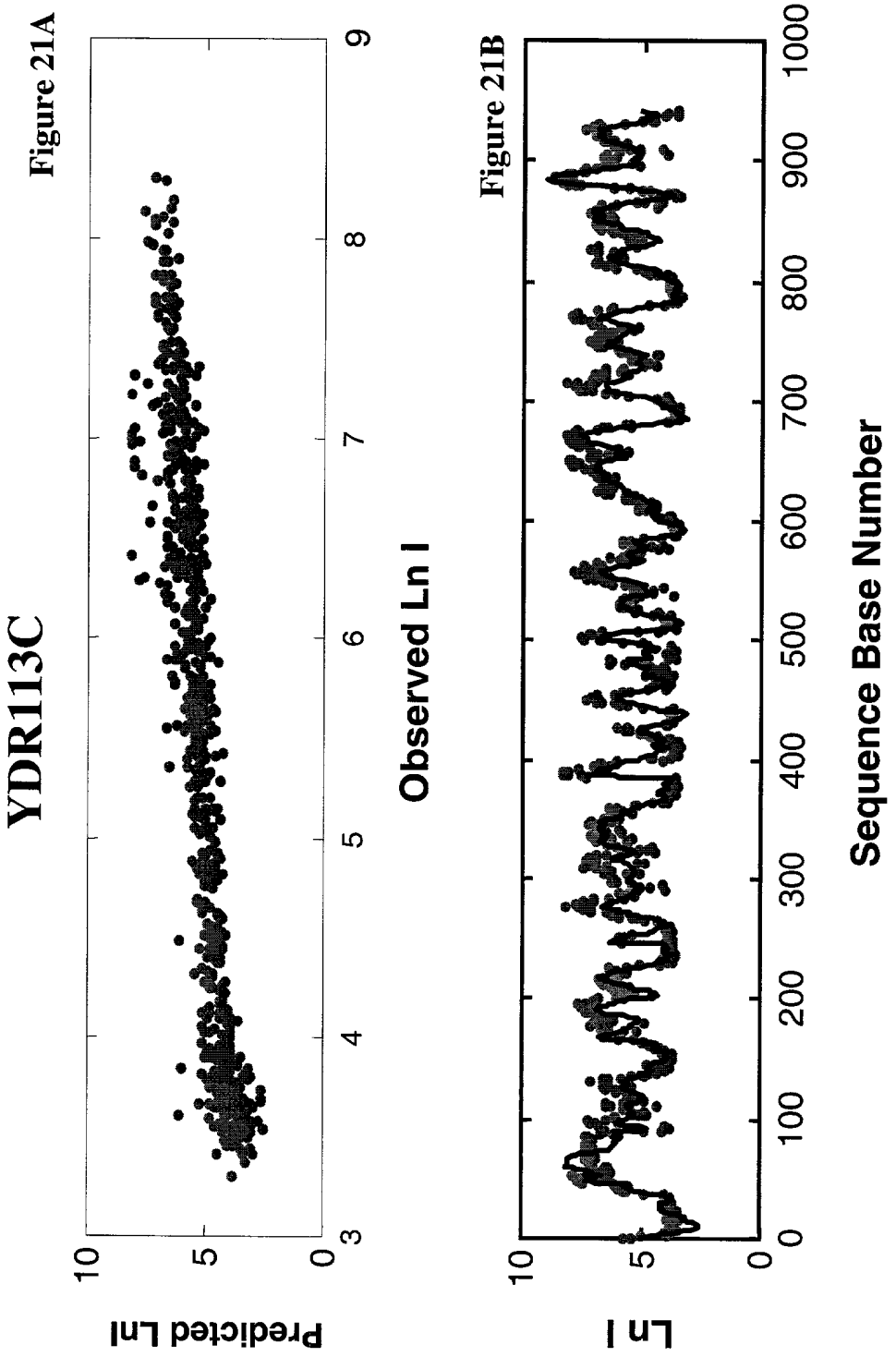


Figure 21A
YDR113C
Predicted Ln I
Observed Ln I
10
5
0
3
4
5
6
7
8
9
Figure 21B
Ln I
Sequence Base Number
10
5
0
0
100
200
300
400
500
600
700
800
900
1000

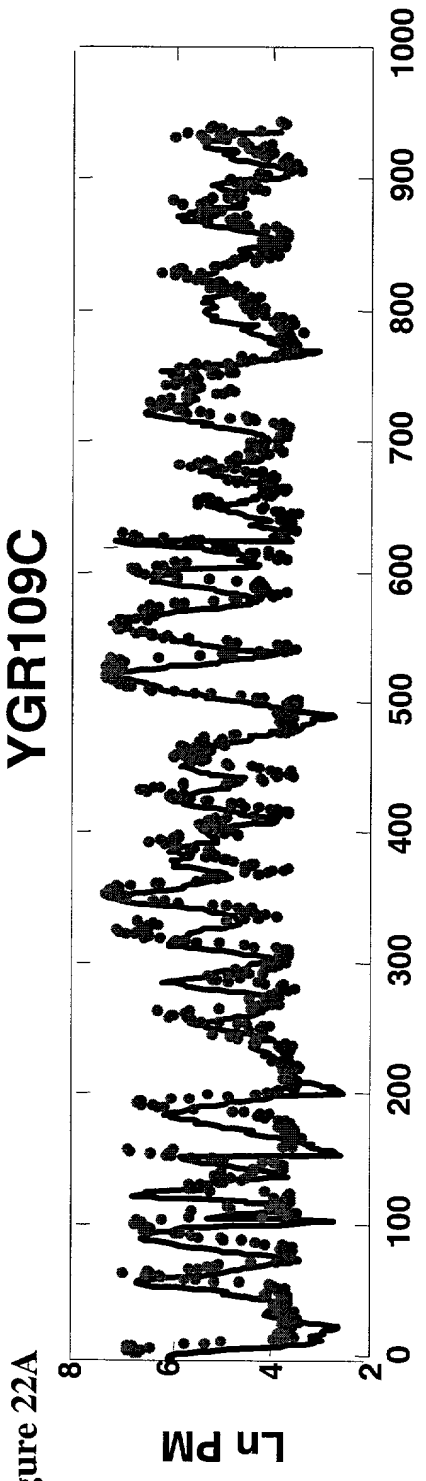


Figure 22A

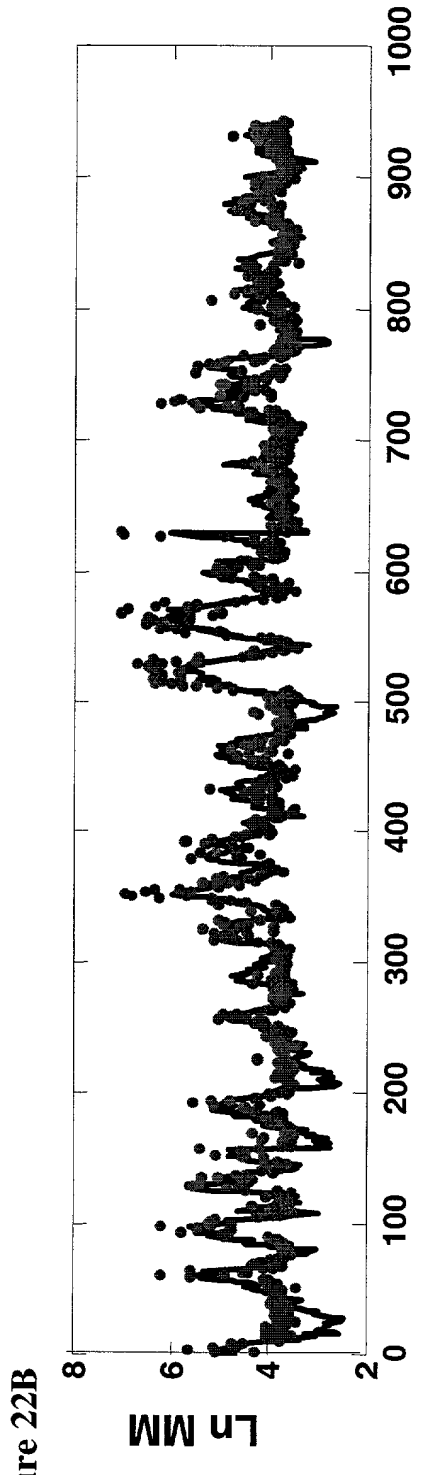


Figure 22B

Ln(Int) at Different Spike Concentrations

8pM

16pM

32pM

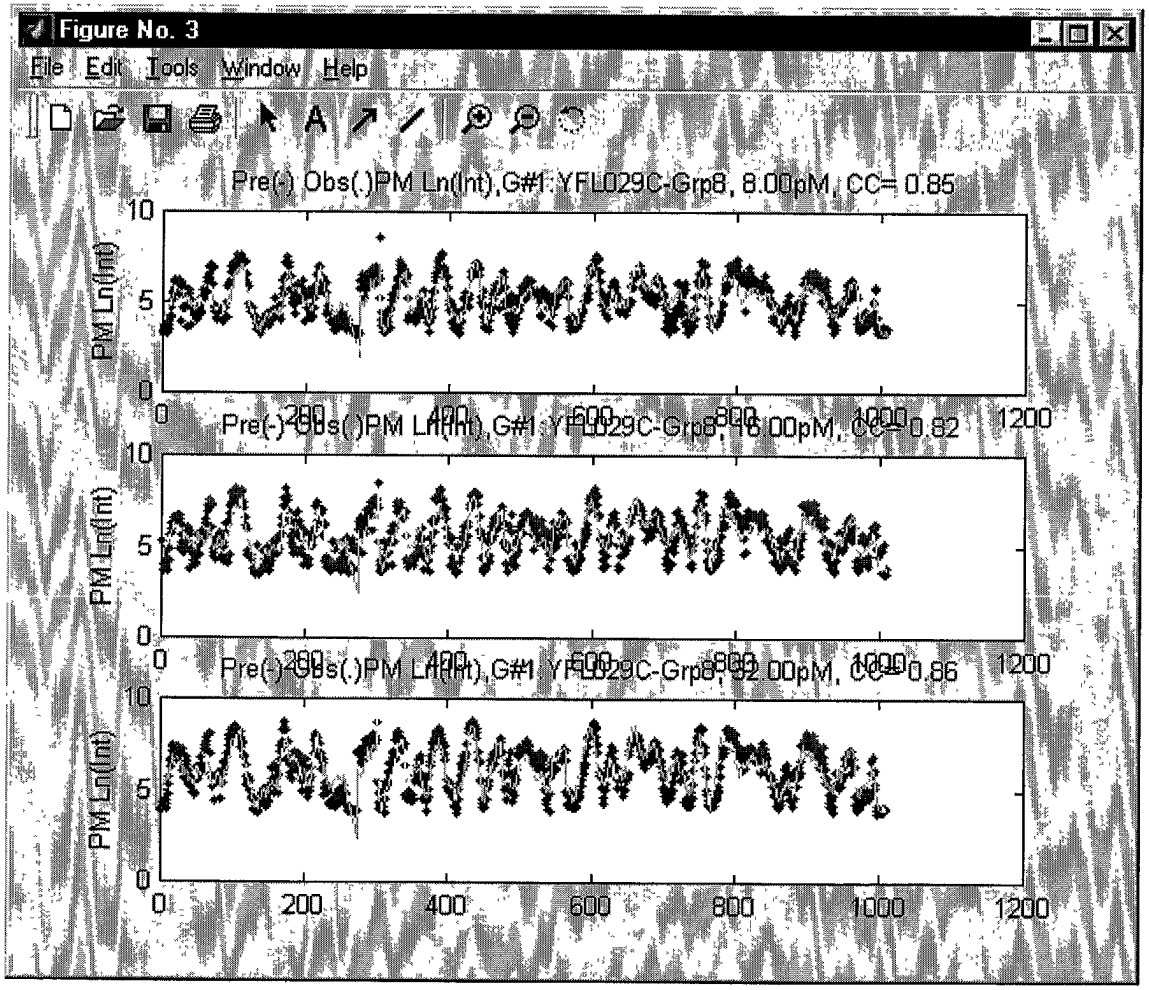


Figure 23

Correlation between Predicted & Observed Ln(Int)'s

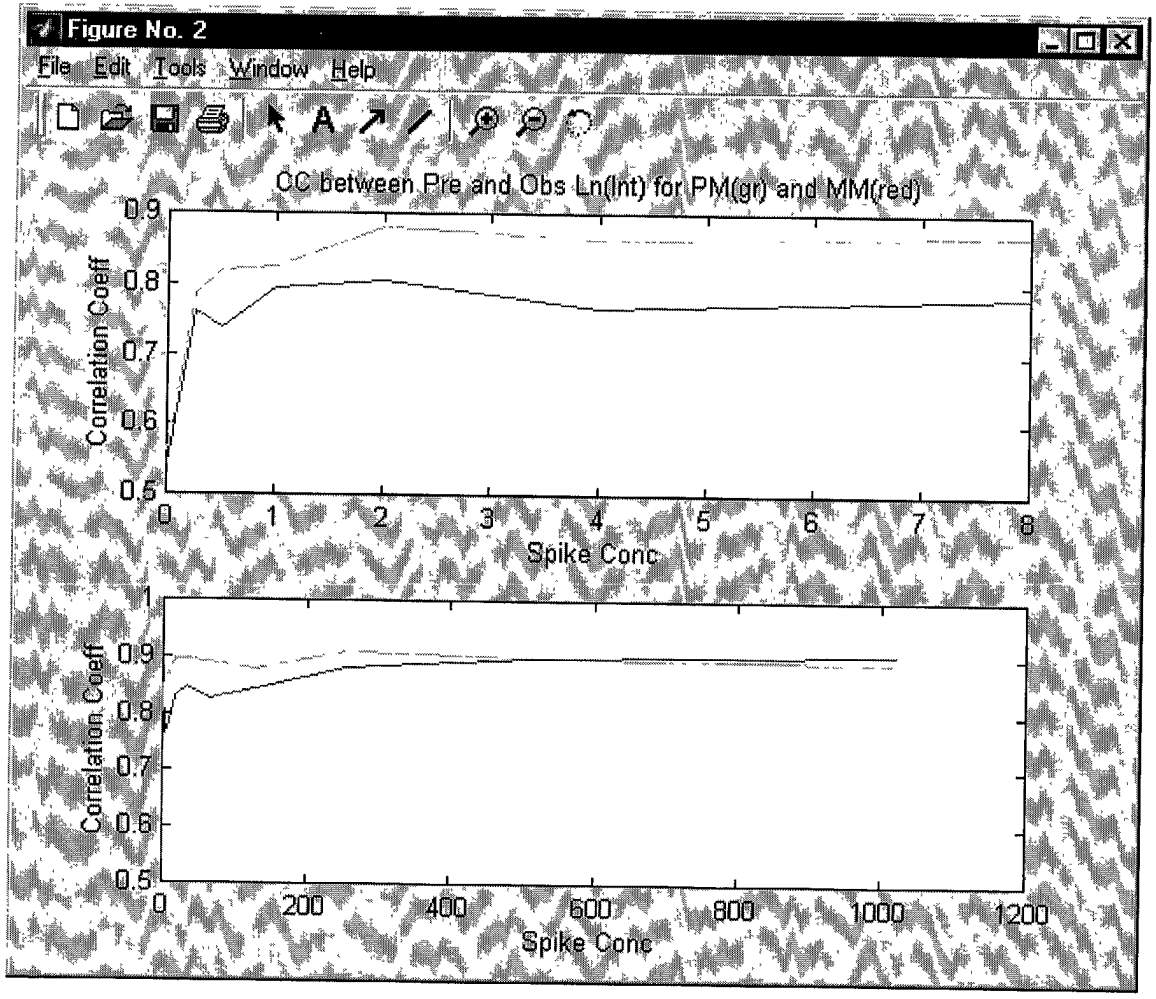


Figure 24

Negative Control: Gene in Wrong Orientation

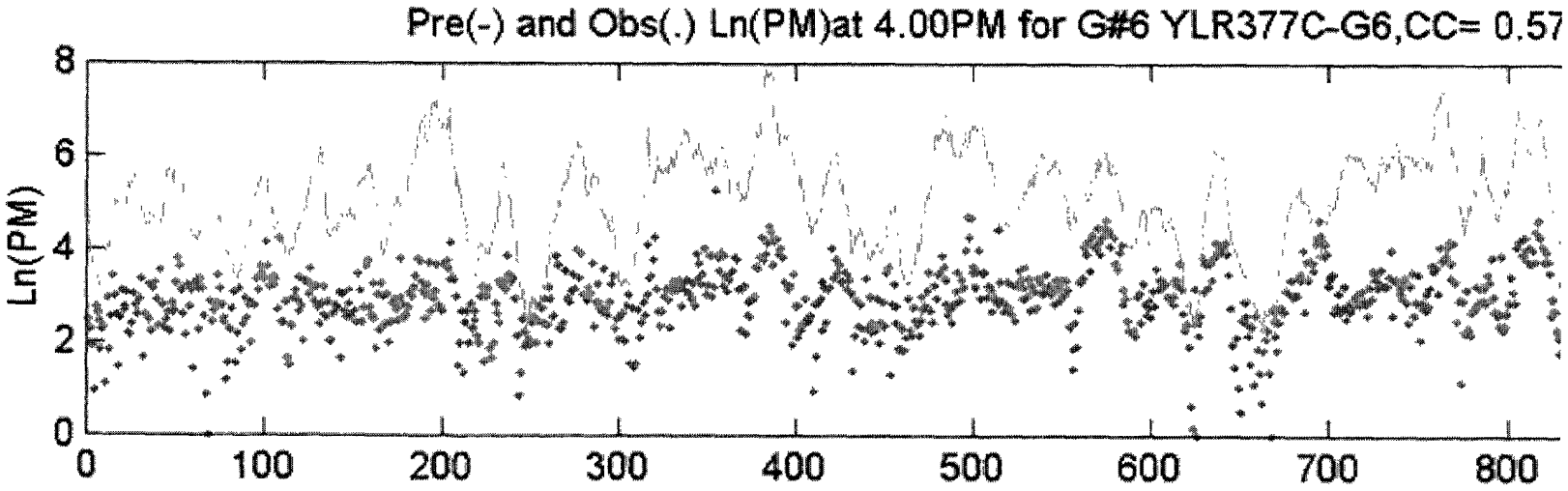


Figure 25

Predicted Observed Slopes

CC=0.42

CC=0.84

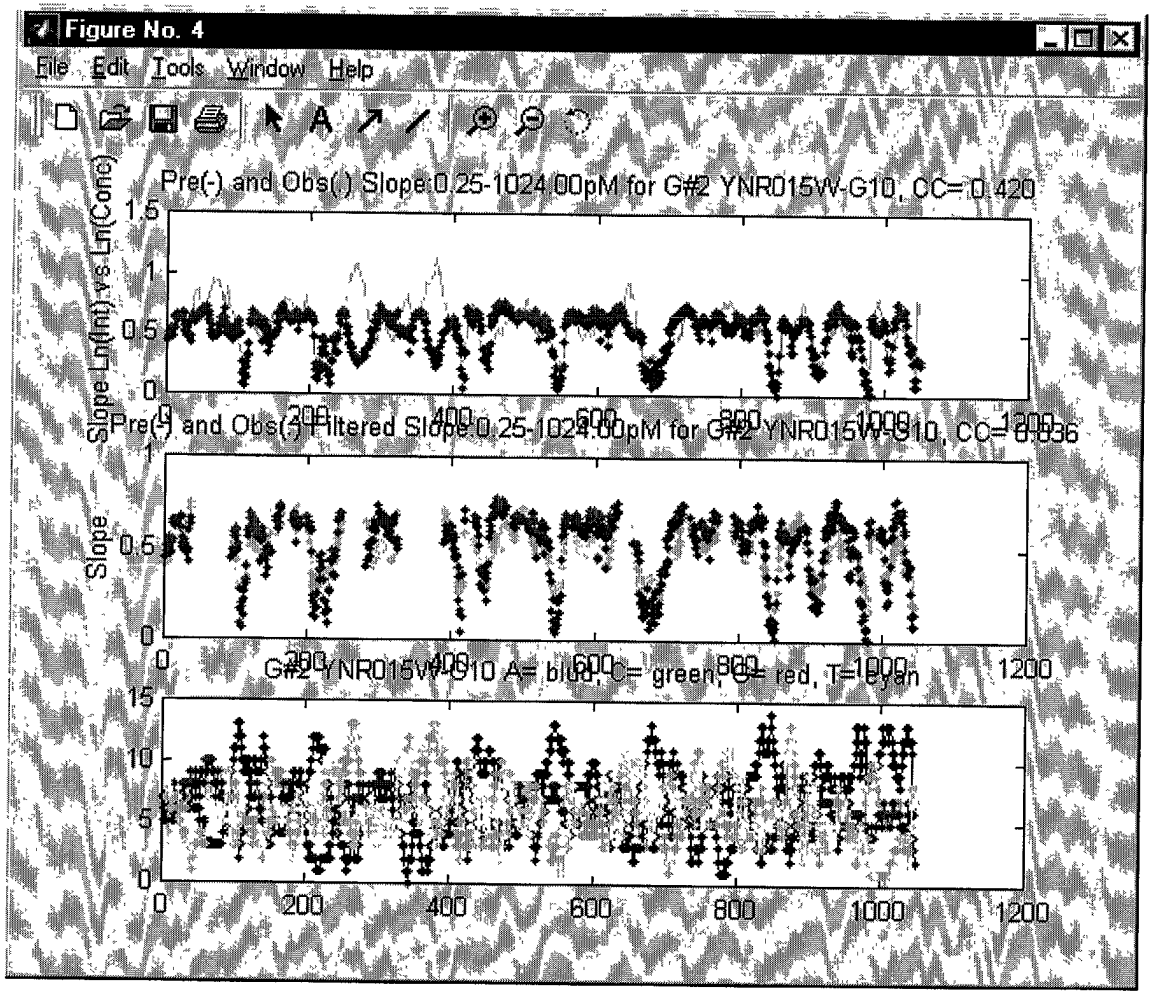


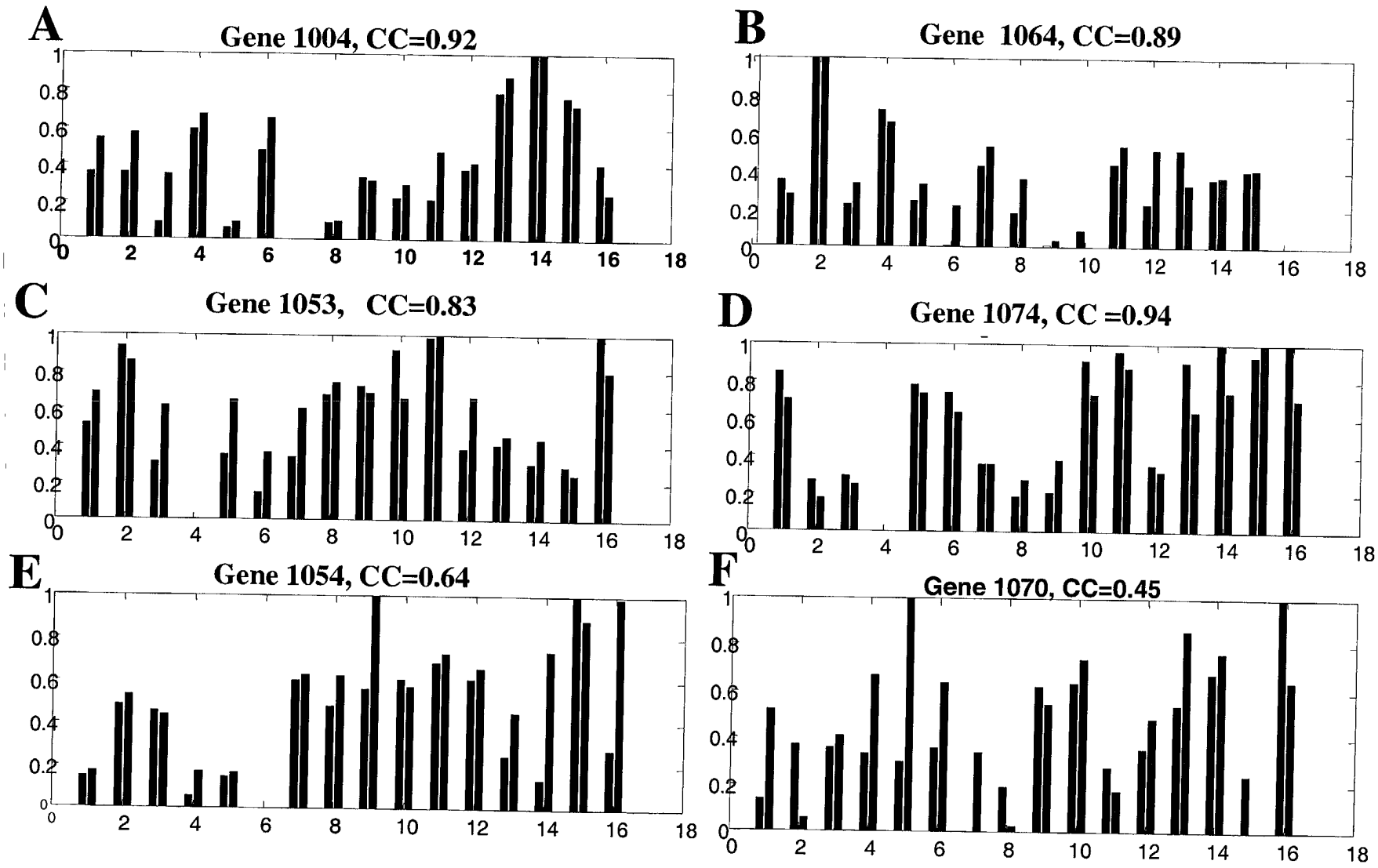
Figure 26

Title: Methods for Selecting Nucleic Acid Probes
Inventor: Hubbell
Attorney Docket No. 3373.1
Sheet 26 of 30

From Yeast to Human

Figure 27

Using Parameters from Yeast Model System to Predict Human_U95A



Predictions for Hu_U95a Probe Sets

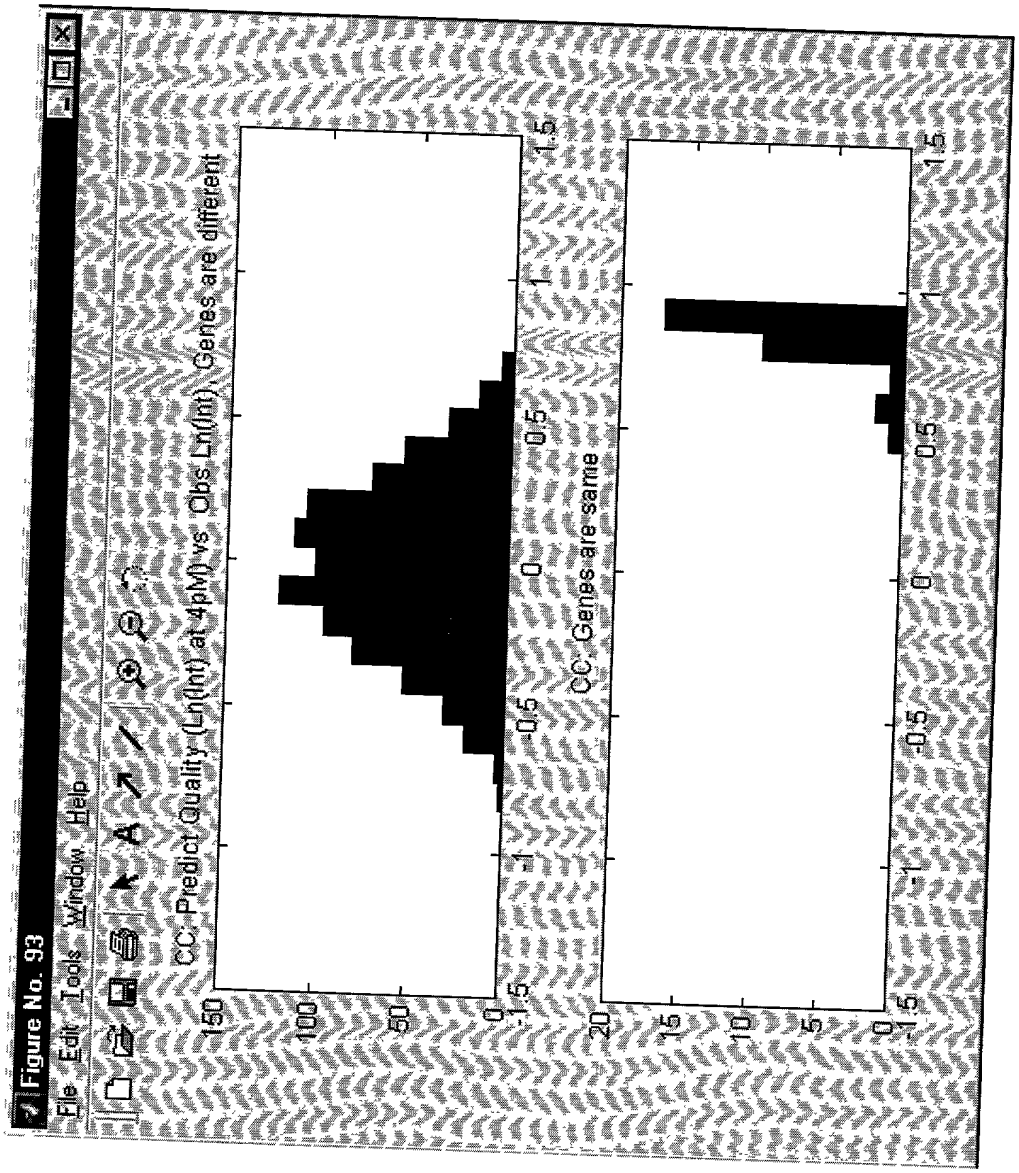


Figure 28

Sixteen Probes Selected By Dynamic Programming Algorithm

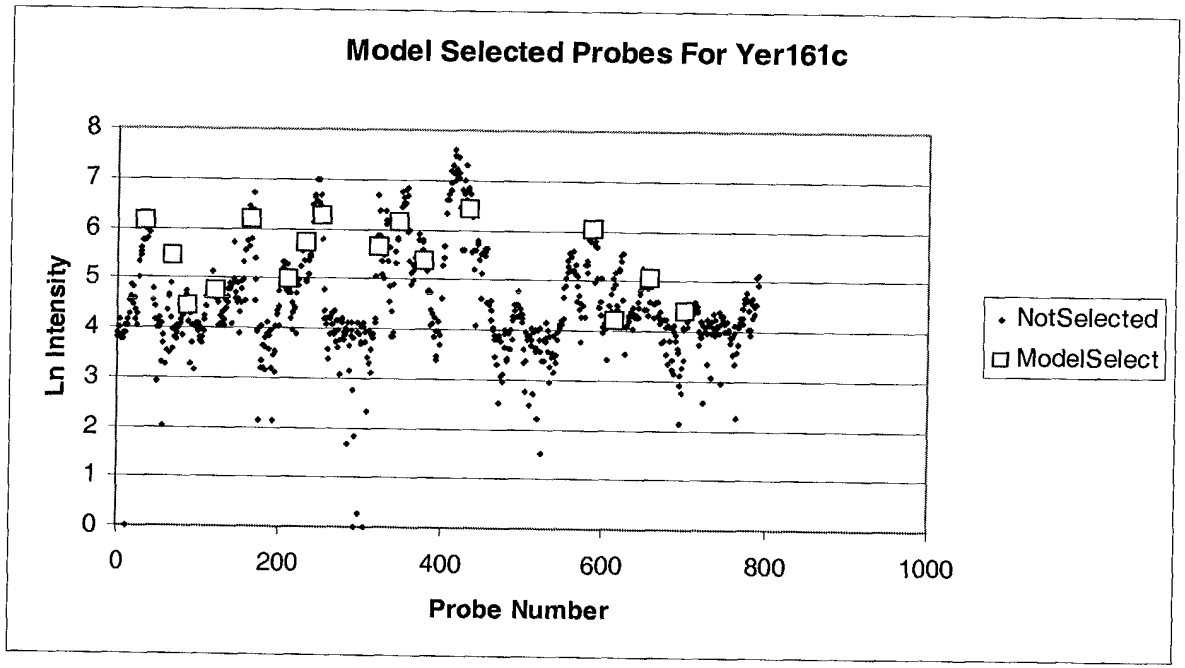


Figure 29

Comparison of AveDiff Values of all Yeast Test Chip Genes:New vs Random vs Rules Selection

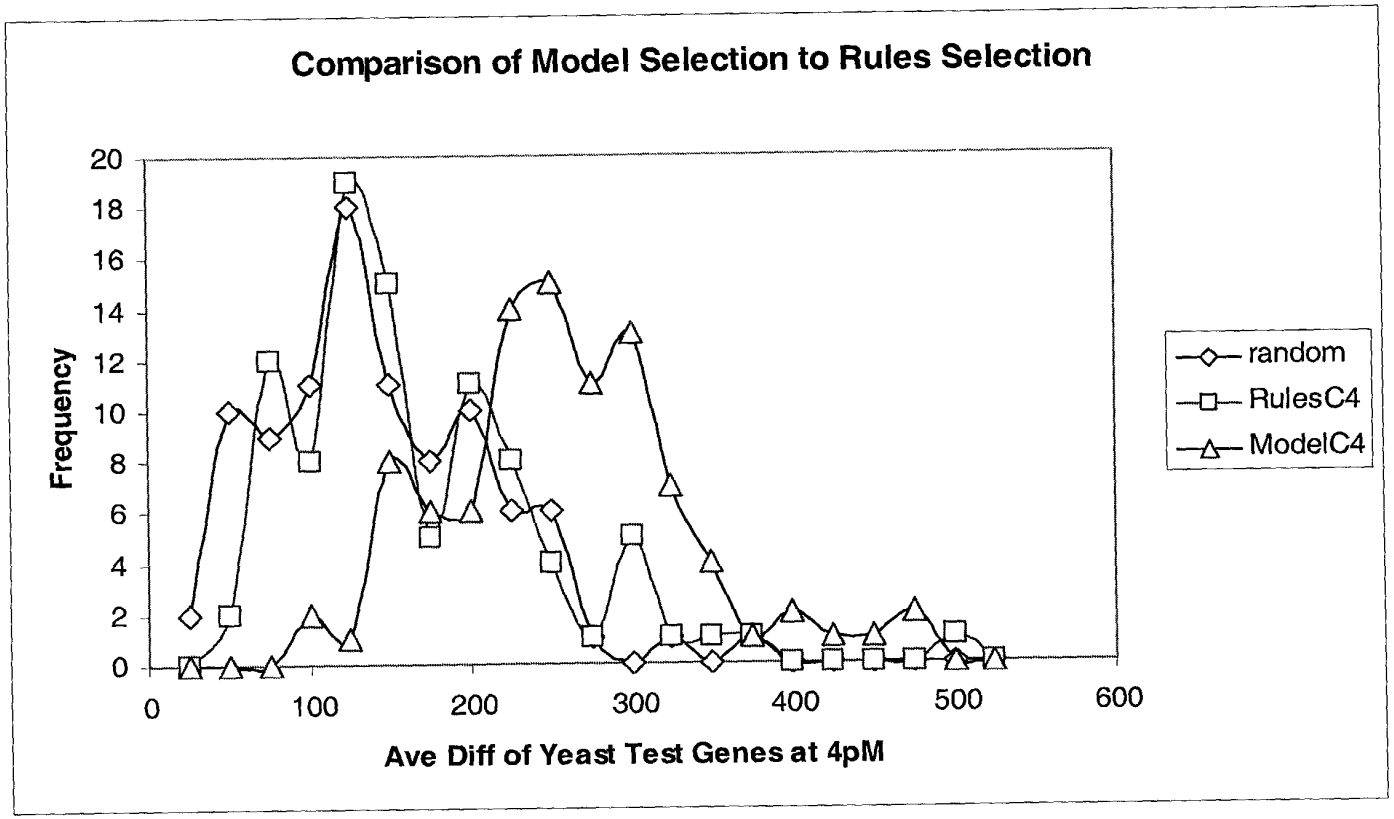


Figure 30